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## Spatial variation of manure nutrients and manure sampling strategy in high-rise laying-hen houses<sup>1</sup>

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Primary Audience: Egg producers, researchers, manure applicators

#### **SUMMARY**

Reliable knowledge of manure nutrient content for intensive animal operations is imperative to development of effective comprehensive nutrient management plans, which will minimize nutrient runoff and pollution of adjacent waterways. The objectives of this study were to evaluate the spatial variation of manure dry-matter (DM), phosphorus (P), and nitrogen (N) content in commercial high-rise laying-hen houses, and to determine the sampling locations and number of samples that will lead to good assessment of nutrient content of manure in the houses. Two side-by-side manure samples were collected from 9 locations in each of 6 high-rise lavinghen houses (18 samples per house) and analyzed for DM, N, and P content. The 9 sample locations were distributed as one-fourth, half, and three-fourths of the building length, with 3 sample locations (every other manure row) per cross-section of the 5 manure rows. The average of DM, N, or P content from the 18 samples per house was used as the reference value for comparison of 8 sampling scenarios. Results showed that duplicate sampling at a location added little to the precision of the data. Manure samples collected crossways across the middle of the house or diagonally across the house in either direction yielded results most similar to the reference value for that house. Hence, when collecting manure samples for nutrient assessment in high-rise laying-hen houses, a single sample collected from every other manure pile across the middle of the building should be sufficient to obtain representative samples of the house and is recommended.

**Key words:** manure sampling, manure nutrients, laying hen

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#### **DESCRIPTION OF THE PROBLEM**

Livestock and poultry manure from confined animal feeding operations is often used as a source of nutrients for agricultural crops. Of egg producers who spread manure onto crop fields, 73% applied that manure based on nutrient requirements for crop production [1]. However, an accurate assessment of the nutrient contents of

<sup>&</sup>lt;sup>1</sup>Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or endorsement by Iowa State University and does not imply the approval to the exclusion of other products that may be suitable.

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manure must be made before sound management decisions with regards to land application of manure are possible. Prior to the analysis of manure nutrient contents, samples must be collected that are representative of and reflect the characteristics of the entire quantity of manure considered. Inaccurate sampling technique will lead to inaccurate nutrient analyses and impede sound management decisions with regards to manure application.

Improper management of manure nutrients can cause extensive pollution and damage to sensitive ecosystems. Over-application of manure may lead to runoff of nitrogen (N) and phosphorus (P) and subsequent nutrient loading into area waterways [2]. Eutrophication is the overproduction of organic matter caused by nutrient imbalances, typically in aquatic systems. Excesses of N or P in a sensitive ecosystem can lead to algal blooms and hypoxia (i.e., low dissolved-oxygen content) contributing to a decrease in biodiversity and fish kills [3, 4]. Agricultural and, to a lesser degree, industrial activities are the primary contributors of excess nutrients into ecosystems around the world [3, 5]. Therefore, proper management of agricultural and specifically manure nutrients is paramount to improving the quality of impaired waterways and to limit nutrient runoff into non-impaired systems.

In addition to the direct pollution of aquatic ecosystems, over application of P-containing fertilizer (e.g., manure) can lead to accumulation of P in the soil, as the corn and soybean crops typically grown in the Midwestern United States require a lower P:N ratio compared to that contained in poultry manure. Application rates based on N requirements for crop production lead to an increase in soil P over time [6]. When soil is over-enriched with P, erosion and sedimentation from fields carry P from agriculturally productive areas to natural environments [5, 7] where the excess P can be harmful, as described previously. Furthermore, P imbalances may persist for years after land-use changes have been implemented [8].

Accurate assessment of manure nutrients allows managers and agronomists to apply manure at the appropriate rates to crop fields, assuring that excess nutrients are not applied to a given parcel of land and that crops will have adequate resources for optimal yields. Because many poultry producers do not own much if any

crop ground on which to spread manure, much of the manure produced in the United States is sold to crop producers based on fertilizer value (i.e., nutrient content). Accurate sampling and analytical techniques are necessary to establish a fair price that reflects the value of the manure for agronomic purposes.

Although tables and reference values for manure nutrient content are published [9, 10], the actual nutrient content of manure varies depending on many different factors. Season, characteristics of the animals' feed, housing scheme, use and type of bedding, and manure handling can all impact manure nutrients, which can be drastically different from published values [11–14]. For example, Peters [15] reported that the N content of solid dairy manure varied between 1.5 and 16.5 g/kg (3 to 33 pounds per ton) for 388 samples submitted to the Wisconsin Soil Testing Labs over a 6-year period. Lorimor and Xin [14] examined the nutrient content of laying-hen manure on 4 farms in Iowa and reported that the average observed N content was 42% lower, P was 60% higher, and K was 58% higher than published manure values for Iowa. Therefore, sampling and laboratory analyses are necessary to establish the actual nutrient content of manure before land application.

Manure samples can be collected from manure storage or during load-out and subsequent land application. Dou et al. [12] reported that sample-to-sample variability was much lower when manure was agitated either in liquid or solid handling systems and the mixing that occurred during load-out of broiler manure was sufficient to decrease sample variability. Similarly, Peters [15] recommended sampling stacked or bedded-pack manure (in this case, dairy manure) during load-out rather than directly from the stack in order to get a representative sample. However, while uniform samples may be obtained during manure load-out, nutrient analyses performed at commercial laboratories cannot be available for determining land application rates. Sampling manure from the storage area, several days before load-out, allows sufficient time for analyses such that the actual manure nutrient content can be used to calculate application rates.

The objective of this study was to evaluate the spatial variation of nutrients (i.e., N and P) as well as dry matter (**DM**) content in

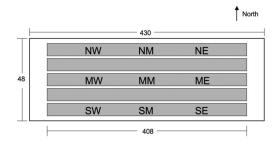


Figure 1. Diagram showing 5 manure rows in gray and nine sampling locations in high-rise laying-hen houses. Two samples were collected at each location to yield 18 samples in total per houses. Abbreviations are as follows: NW = northwest; NM = north middle; NE = northeast; MW = middle west; MM = middle-middle; ME = middle east; SW = southwest; SM = south middle; and SE = southeast. All measurements are in feet (1 foot = 0.3 meters); not drawn to scale.

laying-hen manure in high-rise houses and to determine which sampling locations should be used and how many samples should be collected that would yield representative manure nutrients of the houses.

#### MATERIALS AND METHODS

This experiment utilized 6 commercial highrise laying-hen houses, designated 1 through 6, each containing between 66,800 and 72,600 Hy-Line W-36 hens housed at 61 in²/hen. Houses had 5 cage rows and, therefore, 5 rows of manure. Houses were 48 by 430 feet with 408-foot cage rows and were oriented east to west. Schematic diagrams of the high-rise laying-hen houses are shown in Figures 1 and 2. Hens in 3 of the houses were fed industry-standard diets while hens in the other 3 houses were fed diets that contained

15% corn distiller's dried grains with solubles. Hens in each house were molted once during the 12-month manure-accumulation period and flocks were replaced in 2 of the 6 houses.

Manure samples were collected from the undisturbed manure piles in October immediately before the annual manure removal. Samples were collected at 9 evenly spaced locations throughout each house at one-fourth, onehalf, and three-fourths the length of the house (Figure 1). Crosswise samples were collected at every other manure row—the first, third, and fifth manure rows (i.e., the 2 outside rows and the middle row). Samples from the outside manure rows were collected from the side of the row nearest the sidewall. Samples from the middle row were collected from the south face of the row. Duplicate samples were collected side by side at each of the 9 locations; hence 18 samples were collected per house.

Manure samples were collected using a manure probe that was designed and built by our research group (Figure 3). A 1-in. inside diameter coreless feed auger was placed inside a 1.5-in. diameter galvanized steel pipe. The pipe was 41 in. long and the auger was 43.5 in. long, i.e., 2.5 in. of the auger extended past the bottom end of the pipe. A 1-in. outside diameter, 18-in.-long steel water pipe was attached inside the top of the coreless auger. The water pipe had a 90° bend at the top with a spinning knob, designed for the steering wheel on a farm tractor, added to facilitate turning the auger. To collect a sample, the manure probe was placed mid-way between the peak and the valley of the manure row at an angle perpendicular to the face of the pile aiming at a floor-level center point of the manure row. The auger

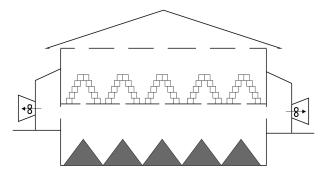
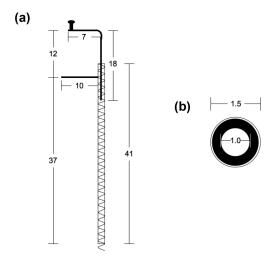


Figure 2. Schematic cross-sectional view of the high-rise laying-hen houses where manure samples were collected.



**Figure 3.** a. Schematic diagram of manure-sampling probe developed by the research group. b. Cross-sectional view of the coreless auger (black ring) inside the 1.5-inch diameter pipe. The auger extends 2.5 inches below the pipe. All measurements are in inches (1 in. = 2.54 cm); not drawn to scale.

was manually turned clockwise while applying gentle pressure to encourage the probe into the manure row. The probe was advanced through the pile until it reached the concrete floor. The probe was not suitable for piles in which the manure was deeper at the sampling location than the 37 inches from the bottom of the probe to the support handle on the side. However, none of the 6 houses sampled contained manure piles that were too deep for sampling with the probe. After sample collection, the probe was withdrawn from the manure pile. Turning the auger counterclockwise extracted the sample from the probe into an appropriate collection vessel (e.g., 5-gallon bucket). The sample volume was approximately one quart (0.95 liters).

Samples were stored at  $-4^{\circ}F$  ( $-20^{\circ}C$ ) in plastic zip-top bags until lyophilization (freezedrying) and grinding through a one mm screen. The DM contents of the manure were determined as the sample weight remaining after lyophilization (freeze-drying). The N [16] and P [17] content of the manure samples were measured and were reported on a dry-matter basis.

#### Statistical Analyses

Statistical analyses were performed using JMP [18]. Least squares means for the 9 loca-

tions were calculated for each response variable (i.e., DM, N, and P); when the main effect of location was significant, the locations were compared pair-wise [19].

Variance components analysis was performed to assess the sources of sampling variability. Variability associated with location within the house (termed "location") and the variability associated with replication within the location (termed "replication") were estimated [20]. The standard error of the mean (SEM) for each variable was reported for different numbers of replications and locations to assess the effect of collecting different numbers of samples on the precision of the estimate.

The variance and SEM for any response variable Y (i.e., DM, N, and P) in a house was calculated according to Equations 1 and 2:

$$Var\left(\bar{Y}\right) = \frac{\hat{\sigma}_L^2}{L} + \frac{\hat{\sigma}_R^2}{L \times R} \tag{1}$$

$$SE\left(\bar{Y}\right) = \sqrt{Var\left(\bar{Y}\right)}$$
 (2)

where  $\bar{Y}$  indicates the mean response in a house,  $\hat{\sigma}_L^2$  and  $\hat{\sigma}_R^2$  represent the estimated variability between locations and replications, respectively, and L and R denote the number of locations and replications, respectively.

Eight sampling scenarios (designated a through h; Table 1) were examined to determine the locations from which manure should be sampled. A reference value was calculated for manure N, P, and DM content for each house by calculating the average value obtained from all 18 samples collected from each house (Table 2). The average N, P, and DM content based on the samples for each scenario, assuming that only those specified samples were collected, was calculated for each house such that a N, P, and DM response was obtained for each scenario and each house. The response for each scenario was reported as the percentage deviation greater than or less than the reference value. Scenarios that yielded deviations nearer zero were considered more desirable than scenarios that yielded large deviations from the 18-sample reference values.

**Table 1.** Manure sampling scenarios in high-rise laying-hen houses.

Scenario	Sample locations <sup>1</sup>	Description
a	ME, MM, MW	Middle row east to west
b	NM, MM, SM	Middle north to south
С	NW, MM, SE	Diagonal northwest to southeast
d	SW, MM, NE	Diagonal southwest to northeast
e	NW, NE, SE, SW	Four corners
f	MM	One location at the middle
g	NW, NM, NE	North row
h	SW, SM, SE	South row

<sup>1</sup>Abbreviations are as follows: NW = northwest; NM = north middle; NE = northeast; MW = middle west; MM = middle-middle; ME = middle east; SW = southwest; SM = south middle; and SE = southeast. Refer to Figure 1 for sampling locations.

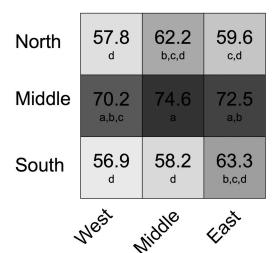
**Table 2.** Average and standard error of the means (in parenthesis) for dry matter (DM), phosphorus, and nitrogen content of manure collected from each of six high-rise laying-hen houses.<sup>1</sup>

	Dry matter	Phosphorus	Nitrogen
Item	(%)	(% of DM)	(% of DM)
House			
1	59.9 (0.9)	2.98 (0.07)	2.87 (0.08)
2	65.9 (3.0)	2.62 (0.07)	3.20 (0.08)
3	80.3 (2.1)	2.69 (0.08)	3.58 (0.10)
4	65.9 (2.1)	2.67 (0.08)	3.40 (0.07)
5	59.8 (2.6)	2.88 (0.07)	3.08 (0.10)
6	53.2 (1.7)	3.03 (0.06)	3.45 (0.07)
Average	64.2 (1.2)	2.81 (0.03)	3.27 (0.04)

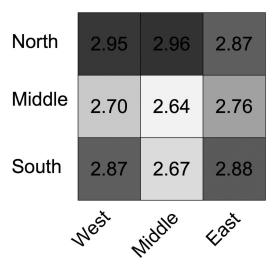
<sup>&</sup>lt;sup>1</sup>These house values are the reference values to which each scenario was compared (Figures 7, 8, and 9).

#### RESULTS AND DISCUSSION

Least squares means for DM, P, and N for each of the 9 locations sampled in 6 high-rise layinghen houses are shown in Figures 4, 5, and 6, respectively. The DM content of samples ranged from 56.9% to 74.6% and higher DM content was observed in the middle manure row compared to the manure along the perimeter walls in the north and south rows. Samples collected at the middle-middle location contained the numerically highest DM content and were not statistically different from the other samples collected along the middle row. Samples collected along the perimeter walls in the north and south rows were not different from each other. During the winter months, moisture in the exhaust

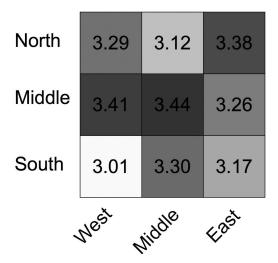


**Figure 4.** Gray-scale plot of the spatial variation of manure dry-matter content (%) under high-rise laying-hen houses; darker gray corresponds to higher values. Values are least-squares means of 6 houses; SEM = 2.4. Values with different letters are different ( $P \le 0.05$ ).



**Figure 5.** Gray-scale plot of the spatial variation of manure phosphorus content (% of DM manure) under high-rise laying-hen houses; darker gray corresponds to higher values. Values are least-squares means of 6 houses; SEM = 0.10; P = 0.25.

air may have condensed on the cooler sidewalls of the houses, resulting in an accumulation of water and lower DM content of the manure in the rows along the perimeter. Furthermore, the moisture gradient in the exhaust air may lead to greater drying ability near the center of the house and lower drying ability (due to more humid air)



**Figure 6.** Gray-scale plot of the spatial variation of manure nitrogen content (% of DM manure) under high-rise laying-hen houses; darker gray corresponds to higher values. Values are least-squares means of 6 houses; SEM = 0.13; P = 0.29.

along the perimeter of the house near the exhaust fans.

The manure P content ranged from 2.65% to 2.96% on a DM basis; however, there were no differences (P = 0.25) among the 9 locations. The N content of manure varied across sampling locations from 3.01 to 3.44% N on a DM basis. As with the P content, there were no significant differences among the locations (P = 0.29)

Estimated variances from the variance components analysis were used to show how the precision of manure contents, expressed as SEM, changes with varying number of locations and replications (Table 3). For the DM measure-

ments, decreasing the number of replications by half (1 rather than 2) and maintaining 9 locations increased the SEM from 2.85 to 3.03. However, decreasing the locations from 9 to 4 and sampling 2 replications per location increased the SEM from 2.85 to 4.28. Therefore, precision was relatively unaffected when half as many samples are collected by decreasing replications but not by decreasing locations. If twice as many samples are considered, the SEM decreases from 2.85 to 2.76 when the number of replications is doubled while keeping the number of locations the same. However, a greater decrease in the SEM can be realized with 36 samples collected at 18 locations with 2 replications.

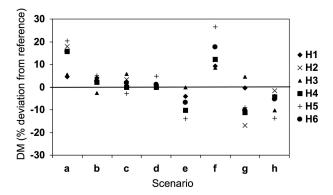
A greater proportion of the variance in the P measurements was due to location rather than replication, as was observed for the DM measurements. The SEM increased from 0.085 to 0.102 when half as many replications were considered (i.e., 1 rather than 2). However, when 4 rather than 9 locations were sampled, the SEM increased from 0.085 to 0.128. Doubling the number of replications from 2 to 4 decreased the SEM from 0.085 to 0.076. However, doubling the number of locations from 9 to 18 decreased the SEM from 0.085 to 0.060. The N measurements revealed a similar trend with more variance associated with location compared to that from replication. Therefore, more total samples collected per house will always give a smaller SEM (more precise value) and collecting samples from more locations within a house will be more beneficial than collecting more samples at each location. When resources are limited,

**Table 3.** Standard error of the mean (SEM) for different numbers of locations or replications (i.e., samples per location) per house.

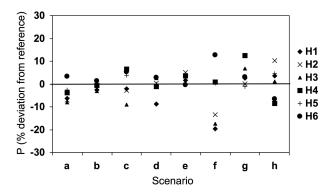
Number of		$SEM^1$			
Locations	Replications	Total samples	Dry matter	Phosphorus	Nitrogen
9	2	18 <sup>2</sup>	2.85	0.085	0.106
9	1	9	3.03	0.102	0.121
4	2	8	4.28	0.128	0.160
9	4	36	2.76	0.076	0.098
18	2	36	2.02	0.060	0.075

<sup>&</sup>lt;sup>1</sup>SEM was calculated according to Equations 1 and 2 using the estimated variance associated with location and replication. The variance estimates for location and replication ( $\hat{\sigma}_L^2$  and  $\hat{\sigma}_R^2$ , respectively, from Equation 1) were as follows: dry matter 63.6 and 19.1; phosphorus 0.037 and 0.057; and nitrogen 0.071 and 0.061

<sup>&</sup>lt;sup>2</sup>18 samples collected at 9 locations with 2 replications was the sampling scheme used to determine the variability associated with location or replication.



**Figure 7.** Deviation of dry matter (DM) measurements from the reference values for each of the 8 sampling scenarios considered in each of 6 high-rise laying-hen houses (refer to Table 1 for scenario configurations).



**Figure 8.** Deviation of phosphorus (P) measurements from the reference values for each of the 8 sampling scenarios considered in each of 6 high-rise laying-hen houses (refer to Table 1 for scenario configurations).

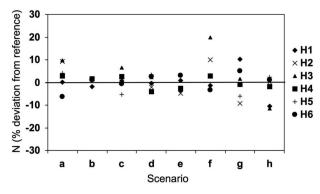


Figure 9. Deviation of nitrogen (N) measurements from the reference values for each of the 8 sampling scenarios considered in each of 6 high-rise laying-hen houses (refer to Table 1 for scenario configurations).

collecting replicate samples at each location may not be necessary to achieve a precise estimate of the manure nutrient contents in a high-rise laying-hen house.

Scenarios were created to determine which configuration of sampling locations gave results

closest to the 18-sample average for each barn. The percent deviations from the reference values for DM, P, and N are shown in Figures 7, 8, and 9, respectively. The DM content of the manure showed the greatest variation across locations and between different sampling

scenarios. Scenarios b, c, and d (middle north to south, diagonal from NW to SE, and diagonal from SW to NE, respectively) yielded manure DM results with the least deviation from the reference 18-sample average, all within 6.0% of the reference. Any of these 3 sampling scenarios would be appropriate to determine the DM content of manure in high-rise laying-hen houses. These 3 scenarios all contain samples collected from both the perimeter and the central part of the house. Therefore, it seems that samples must be collected from manure rows that proportionally cover the variations across the width of the building, e.g., every other row, to adequately represent the nutrient profiles of the manure in highrise houses.

The remaining 5 sampling scenarios yielded inferior results with greater deviations from the reference values. Scenarios a and f consistently overestimated the DM content of the manure by up to 26.6% higher than the reference values. Scenario a involved three samples along the middle row of manure and Scenario f involved one sample collected directly in the middle of the house. These observations indicate that manure in the middle row had higher DM content compared to the average, which agrees well with observations from Figure 4. Scenarios e, g, and h underestimated the DM content of the manure by up to 16.8%. These scenarios involved 4 samples at the corners of the barn, the north outside row, and the south outside row, respectively, indicating that manure along the perimeter of the houses tended to have less DM (i.e., more moisture) compared to the average.

The N and P content of manure, when expressed on a DM basis, showed less variation among sampling scenarios compared to the DM analysis. Scenario f, one sample collected at the middle of the house, provided erratic results with up to a 20% overestimation of the manure N content and a 20% underestimation of the P content. Therefore, one sample is insufficient to reliably determine the nutrient content of manure in a high-rise house. The N and P contents of manure were predicted within 12% of the reference for scenarios g and h while scenarios a through e yielded predictions within 10% of the reference values.

The present research was conducted in highrise laying-hen houses with 5 manure rows each. Different housing schemes may require different sampling procedures. High-rise houses with more rows of manure likely will require the collection of additional samples to represent manure throughout the house. Furthermore, more intensive sampling of broiler or turkey litters in floorrearing systems may be necessary compared to the 3-sample methods proposed for manure in high-rise houses. Dou et al. [12] sampled broiler litter with no mixing and reported that 75 samples were necessary to determine the manure nutrient content with 10% accuracy compared to a reference value calculated as the average of all samples collected at each farm. However, the study did not evaluate manure sampling in high-rise houses.

The method used to collect manure samples can markedly impact the reliability of that sample [13]. The present study used a prototype manure probe to collect all samples, with the objective of determining spatial variation of manure samples within a laying-hen house. Because all samples were consistently collected using the same protocol, the results of nutrient analyses among samples could be compared. However, further research is necessary to assess the quality of manure samples collected with this probe compared to samples collected by traditional means (e.g., grab samples collected during manure load-out or samples collected using a shovel).

### CONCLUSIONS AND APPLICATIONS

- 1. Collecting manure samples from multiple locations in a high-rise house improved the precision of DM and nutrient measurements more so than did the collection of multiple samples from each location.
- 2. The following scenarios yielded results closest to the 18-sample reference values: a) 3 samples bisecting the middle of the house including both outside rows and the center row of manure, b) 3 samples diagonally from NW to SE, or c), 3 samples diagonally from SW to NE.

The manure rows nearer the sidewalls tended to have lower DM content than those near the center of the house, presumably due to moisture gradient in the ventilation air and thus drying ability.

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- 17. The P contents were determined using AOAC method 965.17 adapated to a 96-well plate.
  - 18. JMP. Version 7.0.2, SAS Institute, Inc., Cary, NC.
- 19. Least squares means is the average of observations for each location adjusted for missing values. The least squares means were calculated using a model that included house, location, and house by location as the error term. The least squares means were compared pair-wise using Tukey's test to adjust for multiple comparisons.
- 20. The statistical model included house as a fixed effect and location nested within house as a random effect. Replication within location was the overall error term for the model.

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